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# Platelet and Plasma Serotonin in Patients with Liver Cirrhosis

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**Summary:** To evaluate the role of serotonin in liver cirrhosis, serotonin was determined by high-performance liquid chromatography in plasma, platelets and ascitic fluids from 14 cirrhotic patients. Plasma-free serotonin was within the normal range, but intraplatelet serotonin was significantly low in cirrhosis ($p < 0.001$) and this decrease paralleled the severity of the disease. The concentration of serotonin in ascitic fluids was 12% of the corresponding plasma concentrations.

Our data indicate that serotonin levels are influenced by hepatic injury, but the reasons for these changes are still unclear.

## Introduction

The hypersensitivity to serotonin of isolated mesenteric veins from portal hypertensive rats (1) and the beneficial haemodynamic effects of ketanserin, a selective inhibitor of serotonin$_2$-receptors, in portal hypertensive animals and in patients with liver cirrhosis (2) prompted us to measure the concentrations of serotonin in plasma, platelets and ascitic fluid from cirrhotic patients, to determine whether serotonin might be involved in liver cirrhosis.

## Experimental

Fourteen patients, with diagnoses of liver cirrhosis (8 alcoholic and 6 postnecrotic, mean age ± S.D.: 61 ± 11 years) based on liver biopsy and/or on typical physical and laboratory findings, were studied.

Drugs affecting platelets and serotonin were discontinued at least 8 days prior to blood sampling. Thirteen healthy volunteers matched for age and sex served as normal controls.

Plasma and platelet serotonin was measured by high-performance liquid chromatography using an electrochemical detector.

Venous blood obtained without stasis was collected in 10 ml tubes containing 3 mmol/l sodium EDTA, 50 nmol/l pargyline, 1 mmol/l theophylline, 5 nmol/l imipramine and 33 nmol/l prostaglandin $E_1$.

Platelet-rich plasma obtained by low speed centrifugation (130 *g*, 10 min) was diluted with platelet-poor plasma, obtained by higher speed centrifugation (950 *g*, 10 min) to yield platelet samples containing 110 ± 20 · $10^9$/l. Intraplatelet or plasma serotonin was extracted following the method of *Picard* et al. (3). Briefly, to 0.1 ml of platelet-rich plasma (subjected to ultrasonic treatment for 20 s), or to 1 ml of platelet-poor plasma, were added a fixed amount of internal standard (5-hydroxy-N-methyltryptamine), 1 ml of buffer (pH 11, containing glycine, 0.1 mol/l NaOH and 0.1 mol/l NaCl) and 5 ml of chloroform/1-pentanol (60 + 20, by vol., saturated with water).

Samples were shaken for 10 min and centrifuged (2000 *g*, 10 min). The organic phase was transferred to a second vial containing 400 µl 0.1 mol/l HCl, shaken for 10 min and centrifuged (2000 *g*, 10 min). The aqueous phase was injected into the chromatograph.

The high-performance liquid chromatographic system consisted of a Perkin Elmer Series 2 pump (Perkin-Elmer, Norwalk, CT, U.S.A.), with a Model 7125 Rheodyne valve (Rheodyne, Berkely, CA, U.S.A.); a Nucleosil C18 column (5 µm; 150 × 6 mm inside diameter), (Alltech Europe, Nazareth, Belgium); a Model 5100 A Coulochem detector (ESA Inc., Bedford, MA, U.S.A.) and a Model 561 Hitachi Recorder (Tokyo, Japan).

The mobile phase (1 ml/min, room temperature) consisted of 0.05 mol/l acetate-citrate buffer (pH 4.6) containing 50 ml/l methanol. The average coefficient of variation of the method and the lowest limit of detection have been shown to be 3.5% and 1.2 nmol/l, respectively (4).

Ascites drawn from five patients was processed as described for blood and assayed for serotonin.

Serotonin levels in patients and controls were compared with the aid of a paired *Student* t-test. Statistical differences between patient groups were established by use of one way variance analysis and the *Scheffé* multiple comparison test. Differences were considered significant when the two-tailed p was less than 0.01.

## Results

Platelets from cirrhotic patients contained significantly less serotonin than those from normal controls ($p < 0.001$), while the levels of free circulating serotonin in the two groups were similar (tab. 1). We found no significant correlation between circulating and intraplatelet serotonin levels in patients or in controls, and none of the serotonin measurements correlated with the platelet count, serum albumin, ammonia, transaminase, immunoglobulins and creatinine, or the presence of cholostasis. However, patients with more severe disease — severity was graded by the *Child* criteria (5) — had significantly lower intraplatelet serotonin than patients with milder disease ($p < 0.01$). There were no significant differences in circulating serotonin (fig. 1). Neither circulating serotonin (18.2 ± 15.8 vs 22.1 ± 20.4 nmol/l, mean ± S. D.) nor platelet serotonin (2.5 ± 0.8 vs 2.6 ± 2.2 nmol/$10^9$ platelets) could distinguish alcoholic from postnecrotic cirrhosis. All the ascitic fluids had appreciable amounts of serotonin, representing 12% of the corresponding plasma values (tab. 1). One value, from a haemorrhagic fluid, was 140% of the corresponding plasma level (17.0 and 12.5 nmol/l, ascites and plasma), and this was not included in the calculations of table 1.

Tab. 1. Concentrations (mean ± S. D.) of serotonin in plasma, platelets and ascites from cirrhotic patients and healthy controls.

| | Plasma (nmol/l) | Platelets (nmol/$10^9$ platelets) | Ascites (nmol/l) |
|---|---|---|---|
| Cirrhotics | 19.9 ± 17.0 | 2.5 ± 1.6 (*) | 3.4 ± 1.7 |
| Controls | 15.0 ± 6.2 | 4.6 ± 1.2 | |

(*) $p < 0.001$ (patients vs controls)

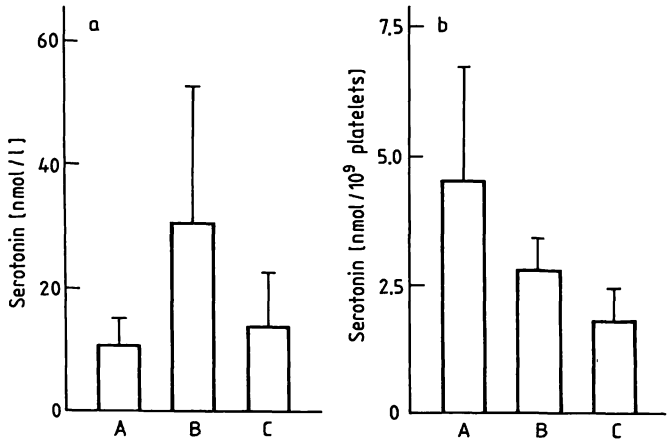


Fig. 1. Circulating (plasma, a) and intraplatelet (b) serotonin levels (mean ± S. D.) from cirrhotic patients grouped upon the *Child-Turcotte* criteria A, B, C.
A indicates the mildest hepatic injury and C the most severe.
The differences in plasma values are not significant. For intraplatelet values A vs. C is significant at $p < 0.01$.

The concentration of serotonin in ascites was independent of the plasma and intraplatelet serotonin contents and of the levels of proteins, glucose, alkaline phosphatase, amylase or urea in the ascitic fluid.

## Discussion

Until now, no data have been published on plasma free serotonin in liver diseases. Low intraplatelet serotonin was previously observed by *Ahtee* et al. in a small number of patients with alcoholic liver cirrhosis, and considered to be due to reduced platelet serotonin uptake (6, 7). However, reduced uptake should lead to increased concentrations of free serotonin in plasma, but free serotonin was found to be within the normal range in the plasma of our patients. However, a number of factors might influence the concentration of circulating serotonin in cirrhotics, e. g. the by-pass of portal blood in systemic circulation, an altered serotonin catabolism due to increased monoaminoxidase activity (8), an abnormal availability of free tryptophan, the precursor of serotonin (9). The wide range of circulating serotonin found in cirrhotics might well be the result of a large number of different contributing factors. Free serotonin levels are thus not representative of the extent of hepatic injury. The low intraplatelet serotonin and, more significantly, the fact that platelet serotonin decreased as the severity of liver disease increased, are difficult to explain. It has been reported that platelet uptake of serotonin is reduced in liver cirrhosis (6, 7), but the mechanism for this is not known, even though metabolic factors or altered serotonin receptors can be hypothesized (7).

The normal concentration of free serotonin (free serotonin is the active form of serotonin) found in cirrhotic plasma does not indicate a role for ketanserin in liver cirrhosis. On the other hand, the observation that platelets containing low serotonin are more responsive to serotonin-induced aggregation in vitro (10) and the abnormal vascular sensitivity to serotonin of mesenteric veins from portal hypertensive rats (1) might indicate a role for such $serotonin_2$ receptor antagonists in liver disorders.

In conclusion, our data indicate that:

1) intraplatelet serotonin levels are influenced by hepatic cirrhosis and

2) intraplatelet but not circulating serotonin is an index of the severity of hepatic damage.

The factors underlying this observation, and the question of whether it can be influenced by ketanserin require further study.

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